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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte HELKE HILLEBRAND,
MARCUS EBNETH, TORGNY NASHOLM,
OSKAR ERIKSON, and MAGNUS HERTZBERG

Appeal 2011-000273
Application 10/593,181
Technology Center 1600

Before DEMETRA J. MILLS, LORA M. GREEN, and
FRANCISCO C. PRATS, *Administrative Patent Judges*.

PRATS, *Administrative Patent Judge*.

DECISION ON APPEAL

This appeal under 35 U.S.C. § 134 involves claims to methods for producing transgenic plants. The Examiner entered a rejection for obviousness.

We have jurisdiction under 35 U.S.C. § 6(b). We affirm.

STATEMENT OF THE CASE

Claims 1-4, 10, and 27 stand rejected and appealed (App. Br. 2).

Claim 1 is representative and reads as follows:

1. A method for producing a transgenic plant comprising:

i) transforming a plant cell with a first expression cassette comprising a nucleic acid sequence encoding a D-amino acid oxidase operably linked with a promoter allowing expression in plant cells or plants, in combination with at least one second expression cassette suitable for conferring to said plant an agronomically valuable trait, and

ii) providing at least one first compound X, which is phytotoxic against plant cells not functionally expressing said D-amino acid oxidase, wherein said compound X can be metabolized by said D-amino acid oxidase into one or more compound(s) Y which are non-phytotoxic or less phytotoxic than compound X, and

iii) treating said transformed plant cells of step i) with said first compound X in a phytotoxic concentration and selecting plant cells comprising in their genome both said first and said second expression cassette, wherein said first expression cassette is conferring resistance to said transformed plant cells against said compound X by expression of said D-amino acid oxidase, and

iv) providing at least one second compound M, which is non-phytotoxic or moderately phytotoxic against plant cells not functionally expressing said D- amino acid oxidase, wherein said compound M can be metabolized by said D- amino acid oxidase into one or more compound(s) N which are phytotoxic or more phytotoxic than compound M, and

v) breaking the combination between said first expression cassette and said second expression cassette and treating resulting said plant cells with said second compound M in a concentration toxic to plant cells still comprising said first expression cassette, and selecting plant cells

> comprising said second expression cassette but lacking said first expression cassette,
>
> wherein said first compound X and said second compound M both comprise a D-amino acid.

The sole rejection before us for review is the Examiner's rejection of claims 1-4, 10, and 27 under 35 U.S.C. § 103(a) as obvious over Signer,[1] Nasholm,[2] Stougaard,[3] and Boeke[4] (Ans. 4-7).

DISCUSSION

The Examiner cited Signer as teaching "a method of generating transgenic plants that utilize both a positive selection marker and a negative selection marker in order to remove the selection markers from the resulting transgenic plants" (Ans. 4). The Examiner conceded, however, that Signer differs from the claimed process in that Signer does not "teach a sequence encoding a D-amino acid oxidase gene for use as either a positive or a negative selection marker" (*id.* at 5).

The Examiner cited Nasholm to meet that deficiency, noting Nasholm's teaching that "D-amino acid oxidase could be used as a positive selection marker with D-alanine and D-serine because D-amino acid oxidase would alleviate the toxicity caused by D-alanine and D-serine (see fourth paragraph on page 35)" (*id.*). The Examiner found that Nasholm also teaches that "D-amino acid oxidase could be used as a negative selection

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[1] WO 01/96583 A2 (published December 20, 2001).
[2] WO 03/060133 A2 (published July 24, 2003).
[3] Jens Stougaard, *Substrate-dependent negative selection in plants using a bacterial cytosine deaminase gene*, 3 THE PLANT JOURNAL 755-761 (1993).
[4] Jef D. Boeke et al., *5-Fluoroorotic Acid as a Selective Agent in Yeast Molecular Genetics*, 154 METHODS IN ENZYMOLOGY 164-175 (1987).

marker with D-isoleucine because applying D-isoleucine to plants expressing D-amino acid oxidase hindered the growth of the transgenic plant with no visible inhibitory effect on wild type plants (see first paragraph on page 36)" (Ans. 5-6).

Based on these teachings, the Examiner concluded that an ordinary artisan would have considered it obvious to modify Signer's process by "utiliz[ing] a construct encoding a D-amino acid oxidase as taught by Nasholm" (*id.* at 6). The Examiner reasoned that a skilled artisan "would have been motivated to do so, because Nasholm et al taught that one transgene (encoding D-amino acid oxidase) could be useful as both a positive and a negative selection marker, and therefore one would only require one transgene rather than two separate selectable marker genes" (*id.*). The Examiner further reasoned that the concept of using a single gene as both a positive and negative selectable marker "was generally known in the art" (*id.*), and cited Stougaard and Boeke as evidence to support that assertion (*id.* at 6-7).

Appellants contend, for a variety of reasons, that the Examiner failed to make a prima facie case of obviousness. After acknowledging the same differences between the claims and Signer as pointed out by the Examiner (*see* App. Br. 4-6), Appellants urge that "none of the teachings of the secondary references remedy the deficiencies of the primary reference Signer (*id.* at 6).

In particular, Appellants contend, the "entire premise of the Examiner's obviousness argument revolves on Boeke allegedly teaching one transgene being used as a dual-functional selectable marker for first identifying transformants and second for excising the marker. (Office Action

dated December 2, 2009, pp. 10, 12)" (*id.* at 7). However, Appellants argue, rather than teaching the use of a single gene as both a positive and negative selection marker using distinct selection media as the rejected claims recite, Boeke "teaches that their system only uses one selection medium comprising one compound (*i.e.* the 5-FOA) that actually acts with the selectable marker" (*id.*; *see also id.* at 9, 14; *also* Reply Br. at 2-4).

Appellants further argue that modifying the Signer reference in the manner posited by the Examiner would change the principles under which the prior art methods operate (App. Br. 10-11). Moreover, Appellants argue, modifying Signer in the manner posited by the Examiner would require either four or five "quantum leaps between the construct of Signer and the claims" (*id.* at 12). Thus, Appellants urge, "neither Signer nor Nasholm provide any teaching or support for the proposed modifications or for any substitutions that would be required to effectuate the modification to Signer proposed by the Examiner" nor would the remaining references cited make up for those deficiencies (*id.* at 13).

Appellants further argue that none of the cited references would have led an ordinary artisan to modify Signer in the manner posited in the rejection (*id.* at 14-16), but would instead have led the artisan in a different direction than the claimed method (*id.* at 17-19). Still further, Appellants urge, the "substitution of D-amino acid oxidase into the method of Signer would result in a different method than that claimed" (*id.* at 16) since Signer explicitly required the use of separate positive and negative selecting genes, despite using the *CodA* gene, which an ordinary artisan would have recognized from Stougaard as both a positive and negative selection marker

(*id.* at 18 ("Signer teaches that two distinct selectable markers are required even if one of these is a potential dual-functional marker.")).

We select claim 1 as representative of the rejected claims. *See* 37 C.F.R. § 41.37(c)(1)(vii).

Appellants' arguments do not persuade us that the Examiner failed to make a prima facie case that claim 1 would have been obvious to an ordinary artisan.

Signer's process of making transgenic plants has the same basic positive and negative selection steps recited in claim 1, including the final step of selecting plant cells that have the gene that confers an agronomically valuable trait to the plant, but which also lack the selectable marker genes (*see* Signer 11 ("The cells that grow on the negative selective medium are cells that have the PS [positive selectable marker] and NS [negative selectable marker] looped-out and contain only the GI [gene of interest] and the AG [additional gene].")) As Signer points out, the GI and AG can be genes that confer agronomically advantageous properties to the plant (*id.* at 8-9).

Signer thus differs from claim 1 in that, rather than using a single D-amino oxidase gene as both the positive and negative selectable marker as claim 1 recites, Signer instead uses a combination of two genes to confer positive and negative selectability to the transfected plant cells, the construct being characterized by the formula GI-PS-NS-GI or GI-NS-PS-GI (*see id.* at 2). As the Examiner explains, however, an ordinary artisan would have recognized that D-amino oxidase was useful as both a positive and negative selection marker, given Nasholm's disclosure that D-amino oxidase could convert D-alanine and D-serine "into accessible N[itrogen] sources and thus

alleviate the toxicity caused by D-alanine and D-serine" (Nasholm 35), and that in contrast D-asparagine, and especially D-isoleucine, "hinder[ed] growth of transgenic plants expressing [D-amino oxidase] with no visible inhibitory effect on wild type plants" (*id.* at 36).

Given these teachings, we agree with the Examiner that an ordinary artisan would have reasoned that Nasholm's D-amino acid oxidase would be suitable as both the positive and negative selectable marker in Signer's process, and would therefore have been prompted to use D-amino acid oxidase as both the positive and negative selectable marker in Signer's process. Accordingly, we also agree with the Examiner that claim 1 would have been prima facie obvious to an ordinary artisan, particularly when further viewed in light of Stougaard and Boeke's teachings that a single gene can serve as both a positive and negative selectable marker.

It may be true, as Appellants argue, that Boeke's first selection step involves the use of a medium that *lacks* an ingredient (uracil) compensated for by the selectable marker transgene, and Boeke therefore actually uses only one selection medium *supplemented* with a compound allowing for selection by the marker, unlike the two selectable compounds required by Appellants' claim 1 (*see, e.g.* App. Br. 7). However, as we understand it, the Examiner's purpose in citing Boeke was to demonstrate knowledge in the art of the concept of using a single selectable transgene for both positive and negative selection, not to somehow force fit Boeke's teachings wholesale into Signer's methods (*see* Ans. 6).

Thus, we are not persuaded that the Examiner's obviousness conclusion is entirely premised on Boeke. Nor do we detect any specific teaching in Boeke that would have led an ordinary artisan, viewing the

teachings of Signer and Nasholm discussed above, away from using D-amino acid oxidase as the positive and negative selectable marker in Signer's process.

We note, as Appellants argue, that Stougaard discloses that *codA* can be used as both a positive and negative selectable marker (Stougaard 755 ("The *codA* marker therefore provides substrate-dependent negative and positive selection, together with cytosine deaminase reporter activity.")). We also note that, despite *codA*'s capacity for use in both positive and negative selection, Signer only cites *codA* as a negatively selectable marker (Signer 8).

We further note that the Examiner has pointed to no teaching in Signer explicitly suggesting that a single gene marker can be used in its methods as both a positive and negative selector. On the other hand, while it may be true that Signer only explicitly teaches using separate positive and negative selectable markers, Appellants have not pointed to any clear teaching in Signer that would have suggested to an ordinary artisan that using a single gene as both the positive and negative selector would be unsuitable, or would fail to work.

We are not persuaded that, in and of itself, Signer's use of only two separate genes as positive and negative selection markers amounts to a teaching against using a single gene in both selection steps, even given Stougaard's recognition that Signer's *codA* could be used for both purposes. Rather, Signer's use of two genes instead of a single one simply demonstrates that Signer's process is different from the process of claim 1 in that respect. As the Supreme Court has noted, however, "the mere existence

of differences between the prior art and an invention does not establish the invention's nonobviousness." *Dann v. Johnston*, 425 U.S. 219, 230 (1976).

As the Supreme Court has also noted, "the [obviousness] analysis need not seek out precise teachings directed to the specific subject matter of the challenged claim, for a court can take account of the inferences and creative steps that a person of ordinary skill in the art would employ." *KSR Int'l v. Teleflex Inc.*, 550 U.S. 398, 418 (2007). Thus, a "person of ordinary skill is . . . a person of ordinary creativity, not an automaton." *Id.* at 421.

Here, we are not persuaded that Signer's failure to explicitly state that a single transgene can be used for both positive and negative selection demonstrates that an ordinary artisan would have lacked a reason to use D-amino acid oxidase as both the positive and negative selectable marker in Signer's process. To the contrary, given the references' teachings, we agree with the Examiner that an ordinary artisan, being a person of ordinary creativity, would have reasonably inferred that a D-amino acid oxidase would be useful as both a positive and negative selectable marker in Signer's process, and would therefore have been prompted to use it in than manner in Signer's process.

Thus, for the reasons discussed, we are not persuaded that arriving at claim 1's process from the cited references would have involved quantum leaps in logic, nor are we persuaded that an ordinary artisan would have failed to arrive at the claimed process from following the references' teachings, nor are we persuaded that the cited references would have led an ordinary artisan in a direction different than the claimed process. Accordingly, as Appellants' arguments do not persuade us that the Examiner erred in rejecting claim 1 as obvious over Signer, Nasholm, Stougaard, and

Boeke, we affirm the Examiner's rejection of that claim over those references.

Claims 2 and 4 fall with claim 1 as they were not argued separately. *See* 37 C.F.R. § 41.37(c)(1)(vii).

Appellants separately argue that claim 10 would not have been obvious over Signer, Nasholm, Stougaard, and Boeke (*see* App. Br. 19; *see also* Reply Br. 4-5). In particular, Appellants acknowledge the general formulae GI-PS-NS-GI or GI-NS-PS-GI of Signer's construct, in which homologous recombination between the direct repeats of the gene of interest eliminates the intervening DNA resulting in a structure with only one copy of the gene of interest (App. Br. at 19).

However, Appellants argue, "Signer teaches that direct repeats of the gene of interest are required in that particular configuration in order to allow for homologous recombination, crossing over, and the elimination of the selectable markers" (*id.* (citing Signer 2, 5, 13; Examples 1-3)). Thus, Appellants urge, "in contrast to the construct of claim 10, in the Signer construct the direct repeats of the gene of interest themselves correspond to the sequences which allow for the elimination of the selectable markers" (*id.*)

Appellants reason, therefore, that "[b]ecause Signer does not teach or suggest a second expression cassette, which is suitable for conferring an agronomically valuable trait, not being localized between the sequences which allow for the specific deletion of the first expression cassette, a *prima facie* case of obviousness has not been established for claim 10" (*id.* at 19-20).

We do not agree that Signer's construct, when modified to contain Nasholm's D-amino acid oxidase gene as the positive and negative selectable marker, would fail to meet the requirements of claim 10. Claim 10 reads as follows:

> 10. The method of claim [1, wherein said first expression cassette for said D-amino acid oxidase and said second expression cassette for said agronomically valuable trait are both comprised in one DNA construct and combination is broken by deletion or excision of said first expression cassette for said D-amino acid oxidase, and] wherein the DNA construct comprises
>
> a) a first expression cassette comprising a nucleic acid sequence encoding a D-amino acid oxidase operably linked with a promoter allowing expression in plant cells or plants, wherein said first expression cassette is flanked by sequences which allow for specific deletion of said first expression cassette, and
>
> b) at least one second expression cassette suitable for conferring to said plant an agronomically valuable trait, wherein said second expression cassette is not localized between said sequences which allow for specific deletion of said first expression cassette and the resulting plant cell or plant is selection marker free.

As Appellants point out, the structure of the construct used in Signer's process is either GI-PS-NS-GI or GI-NS-PS-GI, with GI corresponding to a gene of interest, NS corresponding to a negative selectable marker, and PS corresponding to a positive selectable marker (Signer 2). As Appellants also point out,

> intrachromosomal homologous recombination between the direct repeats in the [genes of interest (GI) in the] genetic construct promotes crossing-over that loops out and eliminates

> all the intervening DNA (in this case the positive and negative selectable marker genes), leaving behind within the construct only a single copy of the gene of interest itself.

(*Id.* at 4.) Thus, substituting the D-amino acid oxidase for the PS-NS or NS-PS positive/negative selectable portion of Signer's construct would result in the D-amino acid oxidase being flanked by sequences which allow for specific deletion of the cassette allowing expression of the D-amino acid oxidase, as claim 10 requires for the first expression cassette.

As to the disputed element, claim 10 requires the second expression cassette to not be localized between the sequences which allow for specific deletion of the first expression cassette. Either of the two repeated genes of interest (GI) of Signer's construct meets this requirement because ultimately, after specific deletion of the selectable marker through intrachromosomal homologous recombination, one of the GI elements will remain in the construct. As the remaining GI element is not excised, it necessarily cannot be localized between the sequences which allow for specific deletion of the cassette expressing the selectable marker. Moreover, as the GI elements are the sequences that allow for specific deletion, they cannot be between themselves.

Thus, while claim 10 requires two distinct expression cassettes, claim 10 does not contain any language excluding the second expression cassette, in this case Signer's gene of interest (GI), from also being the flanking sequences that allow for specific deletion of the first expression cassette. We are therefore not persuaded that Signer's GI elements fail to meet claim 10's requirement that the second cassette not be localized between the sequences which allow for specific deletion of the first expression cassette.

As Appellants' arguments do not persuade us that the Examiner erred in rejecting claim 10 over the cited references, we affirm the Examiner's rejection.

Appellants also separately argue that the Examiner failed to make a prima facie case that claim 3, and its dependent claim 27, would have been obvious over Signer, Nasholm, Stougaard, and Boeke (App. Br. 20; *see also* Reply Br. 5-6).

In particular, Appellants note that claims 3 and 27, like claim 10, recite that the second expression cassette must not localized between the sequences which allow for specific deletion of the first expression cassette, and urge, therefore, that rejection of claims 3 and 27 is in error for the same reasons as claim 10 (App. Br. 20). For the reasons discussed above regarding claim 10, we find this argument unpersuasive.

Appellants also argue that Signer fails to meet the element in claims 3 and 27 requiring the practitioner to induce deletion of the first expression cassette, urging that "Signer does not induce deletion of the selectable markers but rather waits for the recombination to occur naturally and by chance before conducting the second selection, for example through meiosis" (*id.* (citing Signer 2, 12, and Example 1)). In particular, Appellants argue:

> As an analogy, there is clearly a distinction between the passive waiting and allowing labor to occur naturally and run its course as compared to inducing labor or inducing the birth of a child whether with chemicals or by surgical means. Both are directed to the same goal of child birth; however, the method steps for arriving at such an end goal are unmistakably distinguishable.

(Reply Br. 6.)

We do not find these arguments persuasive. Claim 3 depends from claim 1, and the claim language at issue requires the practitioner to perform the following step:

> inducing deletion of said first expression cassette from the genome of said transformed plant cells and treating said plant cells with said second compound M in a concentration toxic to plant cells still comprising said first expression cassette, thereby selecting plant cells comprising said second expression cassette but lacking said first expression cassette.

(App. Br. A-3.)

We acknowledge that Signer's corresponding disclosure teaches that excision of the selectable markers occurs as a result of cell growth and meiosis (Signer 2 ("During both vegetative growth and meiosis, intrachromosomal homologous recombination between the direct repeats in the genetic construct promotes crossing-over that loops out and eliminates all of the intervening DNA, leaving behind within the construct only a single copy of the gene of interest itself.")).

However, the homologous recombination that removes the selectable markers can only occur by culturing the cells on the positive and negative selection media (*see, e.g.*, Signer 13-14 (Examples 1 and 2)). In fact, the explicit objective of Signer's process is to induce excision of the marker genes (*see, e.g.*, Signer 2-3).

Thus, because the recombination events that excise the selectable marker genes require the practitioner to perform the active step of culturing the cells, Appellants' arguments do not persuade us that Signer fails to meet claim 3's inducing step, particularly since claim 3 does not recite any particular action that must be performed to induce the deletion. Moreover,

Appellants point to no evidence suggesting that the claimed process, or the processes described in the cited prior art, are sufficiently analogous to childbirth such that the term "inducing" would be similarly applicable in both scenarios.

As Appellants' arguments do not persuade us that the Examiner erred in rejecting claims 3 and 27 over Signer, Nasholm, Stougaard, and Boeke, we affirm the Examiner's rejection of those claims over those references.

SUMMARY

For the reasons discussed, we affirm the Examiner's obviousness rejection of claims 1-4, 10, and 27 over Signer, Nasholm, Stougaard, and Boeke.

TIME PERIOD

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a).

AFFIRMED

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